

HISTOLOGIC CHARACTERIZATION OF BOVINE  
FETAL LUNG, LIVER, AND KIDNEY

by

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DEDICATION

To Millie and Andy

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## ABSTRACT

## INTRODUCTION

Just as knowledge of normal function is essential for understanding pathologic processes, knowledge of normal structures and their development is essential for interpreting the microscopic appearance of tissues. Most histologic reports deal primarily with completely formed organs and treatment of embryologic or other developmental aspects is substantially ignored. Only organogenesis and early histogenesis receive considerable attention in embryologic reports. Consequently, knowledge of periods between embryologic and adult histologic consideration are grossly insufficient. This gap occurs when fetal changes are taking place rapidly and primitive patterns are converted to mature, complex arrangements.

Bovine abortion is a major economic concern for the livestock industry. Pathologists work daily with microscopic material from aborted fetuses, and reports on normal fetal histology are few and far-between. In fact, reports are essentially nonexistent for the bovine species. Since most organs change constantly and have microscopic differences throughout fetal development, familiarity with normal fetal histology is imperative for evaluating fetal tissues; because of these differences, fetal histology is often difficult to grasp.

The objectives of this investigation were to histologically follow the development of the lung, liver, and kidney in the bovine fetus. Lung, liver, and kidney sections were selected because they are the organs commonly examined for diagnosing abortions. The goal was to provide descriptions that would assist anyone microscopically

studying fetal tissues, particularly those persons attempting to identify abortive lesions.

I. HISTOLOGIC CHARACTERIZATION OF BOVINE FETAL LUNG, LIVER, AND  
KIDNEY: REVIEW OF LITERATURE

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## Lung

Volumes of literature record lung development in man, but few reports describe normal pulmonary development in other animals. Animals studied include dogs and cats,<sup>1,2</sup> mice,<sup>3</sup> rats,<sup>4,5</sup> guinea pigs,<sup>6</sup> rabbits,<sup>7</sup> pigs,<sup>8,9</sup> sheep,<sup>10-12</sup> and monkeys.<sup>13</sup> Most animal investigations, however, were concerned only with a small aspect of development and how it related to man. The human lung was accepted as a model because studies of other mammals followed similar patterns.<sup>14</sup>

Before viability, the lung is essentially a glandular organ with closed air spaces. Around the 24th day of embryonic life, the endodermal-lined gut forms a diverticulum from the caudal portion of the pharynx, thus beginning the pulmonary system. The endodermal tube proceeds to invade surrounding mesenchymal tissue by dichotomous branching. These divisions are accompanied by cartilage at 10 weeks gestation and new bronchi are complete by 16 weeks. However, cartilage continues to appear until the 24th week, reaching the same extent as found at term.<sup>15</sup> Paired segmental arteries arising from the dorsal aorta caudal to the aortic arches accompany the bronchial tree from its gut evagination and end in capillary plexuses in the developing lung.<sup>16,17</sup>

The epithelial mass invading mesenchyme early in embryonic life has a solid, glandular appearance, and canalization first appears around  $4\frac{1}{2}$  months gestation;<sup>16</sup> this concurs with similar periods of gestation in rabbits,<sup>7</sup> lambs,<sup>10</sup> and monkeys.<sup>13</sup> Boyden<sup>13</sup> called it the canalicular stage when columnar epithelium surrounding primitive air sacs became cuboidal and mesenchymal cells condensed to form primitive alveolar septa. The cuboidal lining cells are rich in

intracellular glycogen and differentiate into both Type I and Type II epithelial cells. In addition, these cuboidal cells make up most of the epithelial lung tissue at 6 months gestation.<sup>16</sup>

Between 6 and 7½ months, small, terminal air sacs give way to air spaces with branching patterns characteristic of alveolar ducts. Some epithelial cells flatten and extend the periphery of their cytoplasm, while interstitial tissue decrease and capillaries become incorporated into interstitial septa.<sup>7,14,16</sup>

Alveolar ducts continue to bud during the interval of 7½ months to near term; further septal thinning and epithelial flattening also occur. Kikkawa et al<sup>7</sup> reported that lung maturation in the rabbit proceeded from the center, so peripheral sections had slightly more primitive arrangements. Avery and Fletcher<sup>15</sup> classified epithelial cells and their locations during this period similar to mature lung. Type I alveolar lining cells covered anastomosing capillaries, and Type II great alveolar cells were cuboidal, glycogen-rich, and clustered at junctional sites. Clara cells were also cuboidal and glycogen-rich but lined respiratory bronchioles and alveolar ducts.

Concomitant development of rich pulmonary lymphatics occurred with development of pulmonary circulation. Lymphatic vessels surrounded bronchi down to alveolar ducts and were usually adjacent to pulmonary arteries and veins.<sup>16</sup> Intrapulmonary lymphoid tissue was not detected at any stage of development in the fetal pig.<sup>14</sup>

It must be stressed that architecture of the lung is never entirely uniform at any given stage of development, but general patterns are apparent and usually consistent among mammalian species.<sup>7,14,15</sup>

## Liver

One characteristic unique to all vertebrates is a functional liver in adults. However, they demonstrate unbelievable diversity in development modes before attaining the final structure. Amphibians retain the most primitive hepatic developmental patterns, while mammals are more complex. Many accounts stating developmental differences exist and were summarized by Elias and Sherrick.<sup>18</sup> Although studies record various developmental aspects of the liver in the pig,<sup>19</sup> rat,<sup>20</sup> mouse,<sup>21</sup> rabbit,<sup>22</sup> and primates,<sup>23</sup> only one investigation concerned the bovine and did not involve microscopic descriptions of the fetus.<sup>24</sup> Most reports dealt either with developmental patterns in the embryo or histology of the mature organ, and omitted fetal development. Since higher vertebrates demonstrated striking similarities in final hepatic architecture and most information related to human development, human patterns were emphasized.

Elias and Sherrick<sup>18</sup> reported a large hepatic diverticulum that bulged from the cranioventral wall of the foregut-yolk sac junction early in embryogenesis. The diverticulum protruded into the septum transversum, and the septum was invaded by a plexus that arose from the vitelline veins and communicated with the umbilical veins. Visceral mesothelium also proliferated and extended between vitelline capillaries but was more caudally located. The hepatic muralium, a system of walls, is established by epithelial cells surrounding capillaries that become sinusoids.<sup>25,26</sup> Elias and Sherrick<sup>18</sup> also thought epithelial cells could originate from converted and recruited mesenchyme, similar to chick and pig embryos.

The entire network of inter- and intralobular bile ductules develop from transformed liver cells. Epithelial vesicles develop

between hepatic cells and become separated by connective tissue. These vesicles flatten and proliferate until they anastomose with larger canals in the periportal spaces. Although most ductules were formed during the first trimester, DuBois<sup>26</sup> observed formation in all stages of fetal development.

A sinusoid was defined as a capillary lined by Littoral cells, or Kupffer cells; these cells were flat and demonstrated ability to engulf particulate matter as it flowed through the capillary. Capillaries originated from hepatic trabeculae advancing into lumina of umbilical and vitelline veins and pushing endothelium in front of them.<sup>18</sup> In addition, Elias<sup>27</sup> considered blood flow through the liver to be partially controlled by sinusoids. Kupffer cells were reported to bulge into the lumina of sinusoids and to slow the flow.

Hepatocytes were building blocks for the muralium and different sizes were apparent. Size and volume were dependent upon location of individual cells in the muralium.<sup>18</sup> Liver cells occupying corners of plates were larger than those in the middle of walls, while the smallest cells were adjacent to perforations in the liver plate. Although hepatocyte nuclei were usually large, Elias<sup>25</sup> believed size was related to chromosome numbers. Wilson and Leduc<sup>21</sup> encountered binucleated and multinucleated hepatocytes in mice and Doljanski<sup>28</sup> reported binucleated cells only in humans after birth. Cytoplasmic mass was small and finely granulated in young hepatocytes, but cytoplasm increased when the liver began to accumulate glycogen and lipid reserves.<sup>26</sup>

In man, hemopoiesis reaches its maximum around the 7th month of gestation and then decreased.<sup>18</sup> Hemopoietic cells appeared between endothelium and hepatocyte, that were extravascular in perivascular

mesenchyme.<sup>18,29</sup> Wilson et al<sup>30</sup> believed hemopoietic cells were descendants of proliferating mesothelium, but Thomas et al<sup>31</sup> followed a complete morphologic spectrum showing liver cells transforming into hemopoietic cells.

The muralium structure of the liver is basic and in accordance with a fundamental plan of vertebrate animals. Architectural patterns are established early and, unlike the fetal lung, change little throughout gestation.

### Kidney

There are numerous reports of the different developmental aspects of the human kidney.<sup>32-34</sup> Investigations have been reported on the sheep,<sup>35</sup> cat,<sup>36</sup> mouse,<sup>37</sup> pig and opossum,<sup>38</sup> and rabbit.<sup>39</sup> Developmental patterns were similar in all mammals, except for a few minor species differences. Excellent accounts by DuBois<sup>40</sup> and Potter,<sup>41</sup> stressing human development, were used for basic developmental patterns.

Development of the kidney is considered one of the most complicated and puzzling processes of histogenesis. Most organs evolved as smooth, direct processes from initial enlargements in the embryo, but is not true for the kidney.<sup>41</sup> The permanent kidney, metanephros, would not differentiate unless it was preceded by successive embryonic excretory organs, the pronephros followed by the highly differentiated mesonephros. Reptiles, birds, and mammals possessed a metanephros, while fish and amphibians developed only a functional mesonephros.<sup>40</sup>

The metanephros originated from 2 types of cells that had differing potentialities. One type, the ureteral buds, arose from the mesonephric ducts and divided into ureters, renal pelvis, calyces, and collecting

tubules. Actively growing portions of the buds were called ampullae, while remaining interstitial portions made up the tubules. These tubules were produced from forward growth of the ampullae. The first 3 to 5 generations of tubules from ampullary divisions dilated to form the renal pelvis, and the next 3 to 5 generations dilated to form minor calyces, papillae, and cribriform areas. Collecting tubules were formed from the last 6 to 9 generations of branching tubules.<sup>41,43</sup>

The second cell type that contributed to kidney development was the metanephric blastema. Because it had stromagenic and nephrogenic properties, it was responsible for the nephrons and connective tissue. Early in the embryo blastema, cells formed a zone 3 to 4 cells thick around the ampullae, and as ureteral buds divided, the blastema cells advanced in front of the ampullae. Interstitial portions between tubules were surrounded by differentiated connective tissue left behind after blastema advancement. This pattern of development occurred throughout life of the ampullae. Cells adjacent to interstitial portions of tubules were converted to connective tissue and those immediately next to ampullae were converted to nephrons.<sup>41</sup>

A discrete zone of nephrogenic cells adjacent to each ampulla was formed during ampullary activity. Nephrogenic vesicles were oval masses that formed near the junctions of ampullae and the remainder of the tubules. Vesicles were soon converted to structures resembling the letter S. Upper portions of the S differentiated into a renal corpuscle, while the remainder became tubular.<sup>44</sup> The S then touched the ampulla so communication was established between the nephron and collecting tubule.<sup>44,45</sup>

Immature renal corpuscles are unique. Their capillaries are covered by cuboidal or columnar cells instead of flattened endothelial

cells characteristic of mature glomeruli. Bowman's spaces were also lined by cuboidal cells that are somewhat flattened along the parietal layer.<sup>29,46</sup> The most immature glomeruli are always located just beneath and parallel to the kidney's capsule. This more or less distinct zone was called the nephrogenic or neogenic zone. Vernier and Birch-Andersen<sup>47</sup> studied fetuses up to 20 weeks gestation and reported the entire spectrum of glomerular development from the most immature to well-developed nephrons. They believed structural renal maturation was related to the relative number of mature nephrons.

Kidney development is complicated and progressive sequences are difficult to ascertain because so many involved processes occur simultaneously. However, mammals demonstrate remarkable similarities in fetal development. Good techniques in light microscopy, electron microscopy, and microdissection are important for a good understanding of the kidney.

This review of literature is not intended to be exhaustive. Although the basic interest was in the middle and late fetal periods, a background of embryonic and early fetal periods was necessary to appreciate and understand the more advanced developmental stages. Histologic descriptions are often absent from literature and seemingly irrelevant, but information and processes are compiled for reference to the microscopic features described in this thesis.



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## II. HISTOLOGIC CHARACTERIZATION OF THE BOVINE FETAL LUNG

## INTRODUCTION

Purves<sup>1</sup> stated that onset of respiration was essential for life to continue from intrauterine to extrauterine, and that fetal development must have evolved sufficiently for the successful transition. Viability of newborn humans was often limited by lung maturity;<sup>2,3</sup> other mammalian neonates cannot be considered exceptions.

Valuable information concerning behavior of neonatal and adult lungs was attributed to a better understanding of fetal histology that often provided clues to physiologic and pathologic processes.<sup>4</sup>

This study was undertaken to gather information about normal development of the bovine lung during the middle and late fetal periods.

## MATERIALS AND METHODS

One-hundred twenty-four fetuses were collected from an abattoir in Huron, South Dakota during April and May in 1976. Gestational age was estimated according to the methods described by Hubbert et al.<sup>5</sup> and any fetuses less than 120 days were discarded. All fetuses were removed from the uterus within 30 minutes after the cow was stunned by a captive bolt. A complete necropsy was performed and specimens were collected for bacteriologic, histopathologic, and radial immunodiffusion analysis (Appendix 1).

Lung, liver, and kidney were placed in 10% buffered neutral formalin for histologic examination, trimmed, embedded in paraffin, sectioned at 6  $\mu$ , and routinely stained with hemotoxylin and eosin (H & E).

## RESULTS

Radial immunodiffusion, bacteriologic, and histopathologic results are tabulated and summarized in Appendix 2.

### Histologic Characterization:

A routine method was established for examining lung sections. To eliminate repetition, characteristics were considered consistent throughout gestation unless specifically described. The results are summarized in Table I and illustrated in Fig 1-28.

3½ to 4½ months gestation - Organoid architecture, representing potential air spaces, of the lung was most evident during this period. Future air passages appeared as simple, tubular spaces that branched frequently in loose mesenchymal stroma. Tubular spaces were lined by columnar epithelium with large, basophilic nuclei located adjacent to luminal surfaces. These lining cells, initial epithelium, were distinguished by clear cytoplasm, distinct cell boundaries, and prominent nuclei. Air spaces were separated by thick walls of abundant mesenchyme. Thin interlobular septa, composed of loosely arranged reticular tissue, separated the parenchyma into a lobular pattern. Prominent lymphatics were often present.

Nerves and blood vessels were usually located near large airways. However, small arterioles and capillaries were randomly situated within the mesenchymal stroma, lying between air spaces. Large bronchi were lined by pseudostratified ciliated, columnar epithelium. Cartilage, glands, and goblet cells were present.

Bronchioles were distinguished by a simple ciliated columnar to cuboidal epithelial lining. Goblet cells, mucus glands, and cartilage

were not usually present, and arterioles were often adjacent to bronchioles. Branching air passages began forming alveolar ducts and sacs later in this period. The lining epithelium was cuboidal with a centrally located nucleus in clear cytoplasm, but it was not nearly as prominent as the epithelium lining larger passages.

5 to 6½ months gestation - Original gland-like architecture of the lung became less apparent. Blood vessels began to appear in increasingly close relationship to air passages, that continued to form complex, saccular spaces. Blood capillaries often bulged into distal air passages and thinned out or disrupted the continuity of the lining epithelium. As spaces became more complex, mesenchymal stroma decreased so that only a thin partition remained between air sacs, that was gradually replaced by a thin network of fibers, fibroblasts, macrophages, and capillaries. Conversion from an immature organoid architecture to a complex system of air spaces for respiratory exchange began to become obvious during this period.

Lobules and interlobular septa increased in size, and lymphatic vessels became increasingly prominent within the interlobular septa.

Debris of many types within all air spaces began to appear with greater frequency and in varying quantities: mucus, meconium, keratinized squamous cells and vernix, epithelial cells, and unidentified material. Mucus was basophilic and arranged loosely in a fibrillar pattern, while meconium was amorphous, clumped, greyish to golden-brown, and had distinct borders. Vernix and squamae were lightly eosinophilic, thick, often spiculed, and had pleomorphic and meshing patterns. Epithelial cells were pleomorphic with faint outlines and nuclei that were usually pyknotic but sometimes absent.

7 months to near term - Few characteristics were unique to this period. Conversion to a complex structure was completed. Flattened, inconspicuous epithelium consistently separated vessels from air sacs, or alveolar spaces. Lobules continued to enlarge, but the overall architecture was not altered. Interlobular septa were wide and the lymphatics remained prominent. As term approached, so-called initial epithelium became difficult to find, even in the most terminal or peripheral air spaces. Lymphoid tissue continued not to be present around airways or vessels. Golden-brown meconium was present more often during this time. In general, the parenchyma was similar to that of lung tissue that had not yet inflated with air.

#### DISCUSSION

Estimation of fetal age according to Hubbert et al<sup>5</sup> was considered accurate within  $\pm 5$  days. The procedure was substantiated by mathematical expressions and statistical analysis.<sup>6,7</sup> Because of severe drought conditions, many farmers in South Dakota were required to extensively cull their beef herds. As a result many fetuses from normal, healthy cows became available. Prior and Lancaster<sup>8</sup> reported that fetal weight or composition were not influenced by maternal dietary levels. Only fetuses considered normal after laboratory evaluation were used for histologic characterization.

Histologic observations of lung sections revealed developmental patterns similar to that of other mammals. The lung was gradually transformed from a mass of mesenchymal tissue with endodermal channels to a highly vascularized system of irregular air sacs lined primarily

by simple squamous epithelium. Capillary migration, along with a progressive decline in mesenchymal stroma, and increased terminal air sacs were important stages necessary for efficient extrauterine respiration and existence. Respiratory function would be seriously impeded and independent existence prior to 6 months gestation slight.<sup>9</sup> The absence of lymphoid tissue around airways and blood vessels in significant when assessed in relation to diagnostic evaluation of abortions. Baskerville<sup>4</sup> considered migration of lymphoid cells from lymphoid organs to the lung occurred via blood after antigenic stimulation. He also thought that antigenic stimulation caused some lymphoid cells to develop from precursor mesodermal cells in the lung's connective tissue.

Various types of debris were common, particularly squamae and meconium. Whether this debris is significant depends upon the quantity and extent within air spaces. To be considered important, debris had to nearly fill the spaces. Fetal hypoxia and distress during routine slaughter collection could have been responsible for much of the debris observed.

Although maturation was not uniform throughout the lung at any one time, progression occurred so that early developmental stages coincided well with fetal age estimations. The findings should be of value to pathologists and diagnosticians involved in the evaluation of bovine abortions.

#### SUMMARY

One-hundred twenty-four bovine fetuses, between 3½ months and near term, were collected at a South Dakota abattoir from clinically



healthy cows. Histopathologic, immunologic, and bacteriologic findings indicated that the fetuses were normal and noninfected, and suitable for histologic evaluation.

Histologic data from lung sections were compatible with developmental stages described for other mammals. Organoid architecture was prominent through 4½ months gestation consisting mainly of endodermal channels of initial epithelium-lined spaces within masses of mesenchyme. This gland-like structure began to disappear and was not present by 7 months. During this transition, capillaries migrated next to air spaces and mesenchymal stroma decreased so more complex, saccular spaces were formed. Around 7 months, epithelium flattened, became inconspicuous, and separated capillaries from air sacs. Architectural changes were not prominent during the last 2 months of gestation. However, lobules enlarged and fetal lungs developed striking similarities to adult lungs. Lymphoid tissue was not observed in normal sections, but various types of debris were present, particularly meconium and squamæ. Little significance was placed on debris unless it nearly filled air spaces. Maturation was not consistent at any one time, however general histologic patterns were apparent.

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TABLE 1: Microscopic Features of Fetal Lung Sections

| Fetal Age                                     | 3 1/2 mo | 4 mo    | 4 1/2 mo | 5 mo    | 5 1/2 mo |
|---|----------|---------|----------|---------|----------|
| Case Number                                   | 76X-198  | 76X-200 | 76X-204  | 76X-225 | 76X-197  |
|   | 76X-199  | 76X-205 | 76X-223  | 76X-224 | 76X-226  |
|   | 76X-227  | 76X-228 | 76X-201  | 76X-206 | 76X-211  |
|   | 76X-216  | 76X-222 | 76X-230  | 76X-163 | 76X-190  |
|   | 76X-207  | 76X-208 | 76X-209  | 76X-210 |          |
| Microscopic Characteristic                    |          |         |          |         |          |
| Visceral Pleura                               | +        | +       | +        | +       | +        |
| Arcolar/Elastic Capsule                       | 1        | 1       | 2        | 3       | 3        |
| Loose Reticular/Elastic Interlobular Tissue   | 1        | 2       | 1        | 2       | 2        |
| Vasculature                                   | +        | +       | +        | +       | +        |
| Nerve   | +        | +       | +        | +       | +        |
| Organoid Architecture                         | 4        | 4       | 3        | 3       | 3        |
| Intrapulmonary Bronchi                        | Neg      | +       | +        | +       | +        |
| Pseudostratified Ciliated Columnar Epithelium | Neg      | +       | +        | +       | +        |
| Goblet Cells                                  | Neg      | +       | +        | +       | +        |
| Arcolar Lamina Propria                        | Neg      | +       | +        | +       | +        |
| Mucularia Mucosa                              | Neg      | +       | +        | +       | +        |
| Tubuloalveolar Mucous Glands                  | Neg      | +       | +        | +       | +        |
| Cartilaginous Rings                           | Neg      | +       | +        | +       | +        |
| Bronchioles                                   | +        | +       | +        | +       | +        |
| Simple Ciliated Columnar/Cuboidal Epithelium  | +        | +       | +        | +       | +        |
| Goblet Cells                                  | +        | +       | +        | +       | +        |
| Mucous Glands                                 | +        | +       | +        | +       | +        |
| Cartilaginous Plaques                         | +        | +       | +        | +       | +        |
| Mucularia Mucosa                              | +        | +       | +        | +       | +        |
| Respiratory Bronchioles                       | Neg      | Neg     | Neg      | +       | +        |
| Cuboidal Epithelium                           | Neg      | Neg     | Neg      | +       | +        |
| Lamina Propria                                | Neg      | Neg     | Neg      | +       | +        |
| Smooth Muscle                                 | Neg      | Neg     | Neg      | +       | +        |
| Alveolar Ducts/Sacs                           | Neg      | Neg     | Neg      | +       | +        |
| Ducts   | Neg      | Neg     | Neg      | +       | +        |

TABLE 1: continued

| Fetal Age                                     | 5 mo    |         |         | 5½ mo   |         |         | 6 mo    |         |         | 6½ mo   |         |         | 7 mo    |         |         |         |         |         |         |         |        |        |         |         |         |
|---|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|--------|--------|---------|---------|---------|
| Case Number                                   | 76X-213 | 76X-214 | 76X-217 | 76X-218 | 76X-219 | 76X-221 | 76X-141 | 76X-203 | 76X-212 | 76X-215 | 76X-221 | 76X-140 | 76X-164 | 76X-179 | 76X-187 | 76X-189 | 76X-202 | 76X-172 | 76X-182 | 76X-188 | 76X-92 | 76X-99 | 76X-115 | 76X-131 | 76X-133 |
| Microscopic Characteristic                    |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |        |        |         |         |         |
| Visceral Pleura                               | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | ND      | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Areolar/Elastic Capsule                       | 3       | 2       | 3       | 2       | 2       | 2       | 2       | 2       | 3       | 2       | 2       | 2       | 2       | 2       | ND      | 2       | 3       | 3       | 2       | 2       | 2      | 2      | 2       | 3       | 2       |
| Loose Reticular/Elastic Interlobular Tissue   | 3       | 3       | 3       | 3       | 2       | 2       | 3       | 1       | 2       | 2       | 2       | 2       | 2       | 2       | 3       | 2       | 2       | 2       | 2       | 3       | 2      | 2      | 2       | 2       | 2       |
| Vasculature                                   | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Nerves  | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Organoid Architecture                         | 3       | 2       | 2       | 2       | 3       | 3       | 2       | Neg     | 1       | 2       | 3       | 2       | 1       | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg    | Neg    | Neg     | Neg     | Neg     |
| Intrapulmonary Bronchi                        | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Pseudostratified Ciliated Columnar Epithelium | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Goblet Cells                                  | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Areolar Lamina Propria                        | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Muscularis Mucosae                            | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Tubuloalveolar Mucous Glands                  | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Cartilaginous Rings                           | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Bronchioles                                   | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Simple Ciliated Columnar/Cuboidal Epithelium  | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Goblet Cells                                  | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Mucous Glands                                 | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Cartilaginous Plaques                         | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Muscularis Mucosae                            | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Respiratory Bronchioles                       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Cuboidal Epithelium                           | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Lamina Propria                                | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Smooth Muscle                                 | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Alveolar Ducts/Sacs                           | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +      | +      | +       | +       | +       |
| Debris  | 2       | 1       | 2       | 1       | Neg     | Neg     | Neg     | 1       | Neg     | 1       | Neg     | 2       | 1       | Neg     | 1       | Neg     | 1       | Neg     | Neg     | 1       | 1      | 2      | 1       | 3       | 2       |

TABLE 1: continued

| Fetal Age                                     | 7 mo    |         |         |         |         |         |         |         |         |         | 7 1/2 mo |        |          |         |          |         |          |         |          |        | 8 mo    |         |         |         |         |     |      |     |      |     |
|---|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|--------|----------|---------|----------|---------|----------|---------|----------|--------|---------|---------|---------|---------|---------|-----|------|-----|------|-----|
|   | 7 mo    |         | 7 mo    |         | 7 mo    |         | 7 mo    |         | 7 mo    |         | 7 1/2 mo |        | 7 1/2 mo |         | 7 1/2 mo |         | 7 1/2 mo |         | 7 1/2 mo |        | 8 mo    |         | 8 mo    |         | 8 mo    |     | 8 mo |     | 8 mo |     |
| Case Number                                   | 76X-137 | 76X-142 | 76X-154 | 76X-158 | 76X-161 | 76X-177 | 76X-181 | 76X-192 | 76X-193 | 76X-220 | 76X-93   | 76X-95 | 76X-132  | 76X-145 | 76X-150  | 76X-156 | 76X-159  | 76X-173 | 76X-174  | 76X-97 | 76X-129 | 76X-135 | 76X-139 | 76X-146 | 76X-147 |     |      |     |      |     |
| Micronscopic Characteristic                   |         |         |         |         |         |         |         |         |         |         |          |        |          |         |          |         |          |         |          |        |         |         |         |         |         |     |      |     |      |     |
| Visceral Pleura                               | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Areolar/Elastic Capsule                       | 2       | 2       | 1       | 2       | 3       | ND      | 2       | 3       | 2       | 1       | 3        | 3      | 2        | 2       | 2        | 2       | 2        | ND      | 2        | 2      | 3       | 2       | 1       | 1       | 2       |     |      |     |      |     |
| Loose Reticular/Elastic Interlobular Tissue   | 2       | 2       | 2       | 2       | 2       | 2       | 2       | 3       | 3       | 1       | 2        | 2      | 3        | 2       | 2        | 3       | 2        | 2       | 2        | 2      | 3       | 2       | 2       | 1       | 1       |     |      |     |      |     |
| Vasculature                                   | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Nerve   | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Organoid Architecture                         | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg      | Neg    | Neg      | Neg     | Neg      | Neg     | Neg      | Neg     | Neg      | Neg    | Neg     | Neg     | Neg     | Neg     | Neg     | Neg | Neg  | Neg | Neg  | Neg |
| Intrapulmonary Bronchi                        | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Pseudostratified Ciliated Columnar Epithelium | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Goblet Cells                                  | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Areolar Lamina Propria                        | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Muscularis Mucosae                            | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Tubuloalveolar Mucous Glands                  | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Cartilagenous Rings                           | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Bronchioles                                   | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Simple Ciliated Columnar/Cuboidal Epithelium  | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Goblet Cells                                  | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Mucous Glands                                 | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Cartilagenous Plaques                         | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Muscularis Mucosae                            | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Respiratory Bronchioles                       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Cuboidal Epithelium                           | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Lamina Propria                                | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Smooth Muscle                                 | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Alveolar Ducts/Sacs                           | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +        | +      | +        | +       | +        | +       | +        | +       | +        | +      | +       | +       | +       | +       | +       | +   | +    | +   | +    | +   |
| Debris  | 2       | 1       | 2       | 3       | 1       | Neg     | 1       | 2       | 1       | Neg     | 1        | 1      | 3        | 1       | 1        | 1       | 1        | 1       | 1        | Neg    | 2       | 2       | 1       | 1       | 1       | 1   | 1    | 1   | 1    | 1   |

TABLE 1: continued

| Fetal Age                                     |  | 8 mo | 8½ mo | NT |
|---|--|------|-------|----|
| Cane Number                                   |  |      |       |    |
| Micronscopic Characteristic                   |  |      |       |    |
| Visceral Pleura                               |  |      |       |    |
| Arcolar/Elastic Capsule                       |  |      |       |    |
| Loose Reticular/Elastic Interlobular Tissue   |  |      |       |    |
| Vasculature                                   |  |      |       |    |
| Nerves  |  |      |       |    |
| Organoid Architecture                         |  |      |       |    |
| Intrapulmonary Bronchi                        |  |      |       |    |
| Pseudostratified Ciliated Columnar Epithelium |  |      |       |    |
| Goblet Cells                                  |  |      |       |    |
| Arcolar Lamina Propria                        |  |      |       |    |
| Macularia Nucosae                             |  |      |       |    |
| Tubuloalveolar Nucous Glands                  |  |      |       |    |
| Cartilagenous Rings                           |  |      |       |    |
| Bronchioles                                   |  |      |       |    |
| Simple Ciliated Columnar/Cuboidal Epithelium  |  |      |       |    |
| Goblet Cells                                  |  |      |       |    |
| Nucous Glands                                 |  |      |       |    |
| Cartilagenous Plaques                         |  |      |       |    |
| Macularia Nucosae                             |  |      |       |    |
| Respiratory Bronchioles                       |  |      |       |    |
| Cuboidal Epithelium                           |  |      |       |    |
| Lamina Propria                                |  |      |       |    |
| Smooth Muscle                                 |  |      |       |    |
| Alveolar Ducts/Sacs                           |  |      |       |    |
| Elastin                                       |  |      |       |    |

TABLE 1: continued

| Fetal Age | Case Number | Microscopic Characteristic                    | 76X-103 | 76X-104 | 76X-105 | 76X-106 | 76X-107 | 76X-108 | 76X-109 | 76X-110 | 76X-111 | 76X-112 | 76X-113 | 76X-114 | 76X-116 | 76X-143 | 76X-144 | 76X-148 | 76X-152 | 76X-180 | 76X-183 | 76X-185 | 76X-191 | 76X-195 |
|-----------|-------------|---|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
|           |             | Visceral Pleura                               | +       | +       | +       | +       | +       | ND      | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Arterial/Elastic Capsule                      | 2       | 2       | 2       | 2       | 2       | ND      | 2       | 2       | 2       | 1       | 2       | 1       | 2       | 2       | 2       | 2       | 1       | 2       | 2       | 2       | 2       | 1       |
|           |             | Loose Reticular/Elastic Interlobular Tissue   | 2       | 2       | 2       | 2       | 2       | 2       | 2       | 2       | 2       | 2       | 2       | 2       | 3       | 2       | 2       | 2       | 2       | 2       | 2       | 2       | 2       | 2       |
|           |             | Vasculature                                   | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Nerves  | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Organoid Architecture                         | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     | Neg     |
|           |             | Intrapulmonary Bronchi                        | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Pseudostratified Ciliated Columnar Epithelium | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Goblet Cells                                  | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Arterial Lamina Propria                       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Mucularia Mucosa                              | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Tubuloalveolar Mucous Glands                  | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Cartilagenous Rings                           | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Bronchioles                                   | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Simple Ciliated Columnar/Cuboidal Epithelium  | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Goblet Cells                                  | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Mucous Glands                                 | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Cartilagenous Plaques                         | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Mucularia Mucosa                              | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Respiratory Bronchioles                       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Cuboidal Epithelium                           | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Lamina Propria                                | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Smooth Muscle                                 | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Alveolar Ducts/Spaces                         | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       | +       |
|           |             | Membranes                                     | 1       | 1       | 1       | 1       | 2       | 1       | 2       | 1       | 1       | 3       | 1       | Neg     | 1       | Neg     | 1       | 1       | 2       | Neg     | 1       | 1       | 2       | 2       |

The capsule and pleura of the lung are included in these photomicrographs. Note the capsule consists of coarse areolar connective tissue rich in elastic fibers. The most striking feature is the change in the overall pattern from an organoid architecture to a complex, saccular arrangement of potential air spaces.

Fig 1-The tubular spaces are lined by initial epithelium and separated by thick walls of mesenchymal stroma. Arterioles are present within the stroma between air spaces (arrow). 76X-204 (3½ months); H & E stain.

Fig 2-Note how branching air passages form air spaces reminiscent of alveolar ducts and sacs. Mesenchymal stroma decreases and the saccular spaces are separated only by thin interstitial spaces. 76X-163 (5 months); H & E stain.

Fig 3-Alveolar partitions continue to thin out and initial epithelium is difficult to find. 76X-177 (7 months); H & E stain.



Fig 1



Fig 2

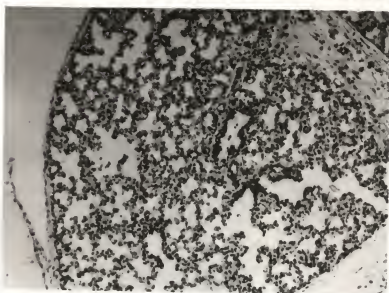
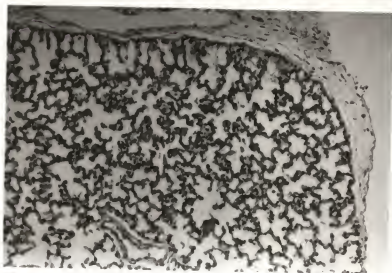


Fig 3



The next 5 photomicrographs demonstrate potential air spaces at higher magnifications.

Fig 4-The tubular spaces here are lined by columnar to cuboidal initial epithelium. These lining cells have clear cytoplasm, distinct cell borders, and prominent nuclei; the lumina are empty. 76X-204 (3½ months); H & E stain.

Fig 5-Original organoid architecture is not apparent in this section. Tubular spaces have branched into a saccular architecture and lining epithelium is not visible. Capillaries begin to bulge into air spaces, disrupting the lining continuity. 76X-163 (5 months); H & E stain.

Fig 6-Interstitial spaces continue to thin. 76X-177 (7 months); H & E stain.

Fig 4

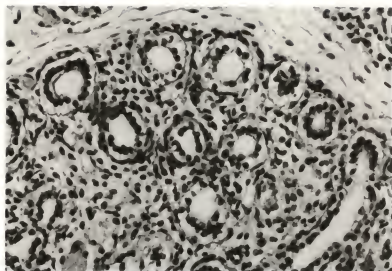


Fig 5

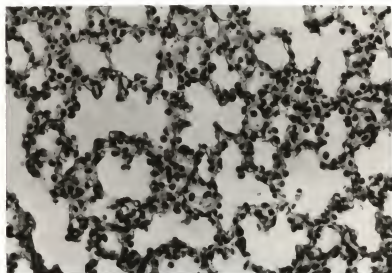


Fig 6

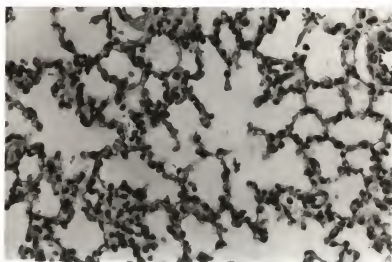


Fig 7-This is a higher magnification of maturing parenchyma.

76X-97 (8 months); H & E stain.

Fig 8-Note bronchiole lined by cuboidal epithelium that is only reminiscent of initial lining epithelium. 76X-111 (near term);

H & E stain.

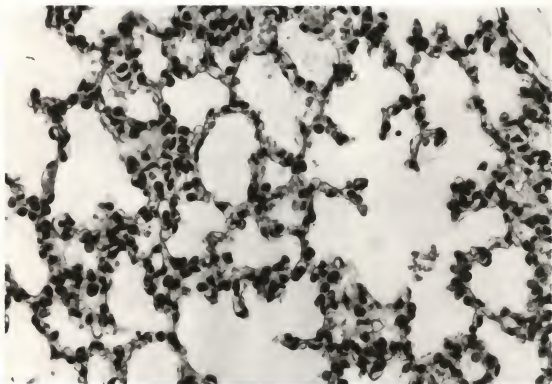


Fig 7

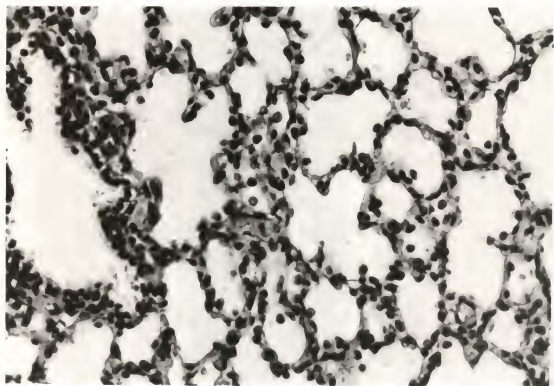


Fig 8

These photomicrographs represent smaller conductive portions of the lung.

Fig 9-This is a tertiary bronchus with pseudostratified ciliated, columnar epithelium. Goblet cells and lamina muscularis mucosae are present, while mucous glands and cartilage are absent.

Fig 10-Bronchioles are smallest divisions of the nonrespiratory portions of the lung. The lamina epithelialis is usually simple cuboidal but may be columnar in more proximal bronchioles. Cilia are present in this bronchiole, however, they diminish in distal bronchioles. It is important that cilia extend further down the respiratory tree than glands. 76X-111 (near term); H & E stain.

Fig 11-Various conductive portions of the lung are apparent here. Note differences in lining epithelium and mural constituents of the large bronchus (L), tertiary bronchus (T), and bronchioles (B). Cartilage is present between the bronchi. 76X-204 (3½ months); H & E stain.

Fig 9

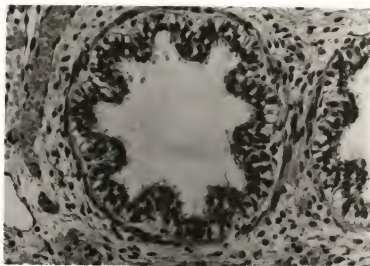


Fig 10

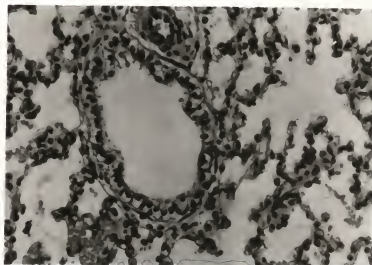


Fig 11

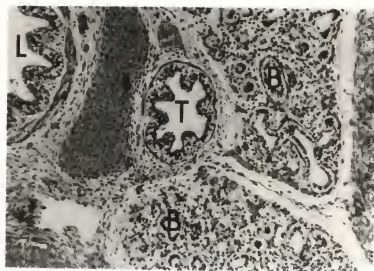


Fig 12-This is low power of a terminal bronchiole entering into an alveolar duct. The bronchiole is lined by cuboidal epithelium and is devoid of goblet cells and cartilage. 76X-97 (near term); H & E stain.

Fig 13-Entrance of a respiratory bronchiole into an alveolar duct at high power is present. Note the arteriole lying adjacent to the bronchiole. Cuboidal cells of the bronchiolar walls are interrupted by outpocketing alveoli. Lamina propria is indistinct, but fine collagenous and elastic fibers support lining cells. 76X-144 (near term); H & E stain.

Fig 14-These are distal pathways of the lung. Alveolar sacs are surrounded by alveoli. 76X-111 (near term); H & E stain.



Fig 12

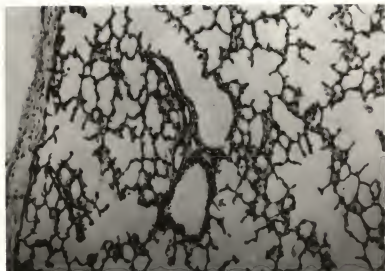


Fig 13

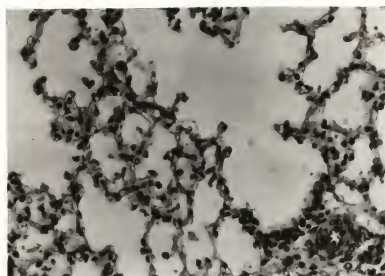


Fig 14

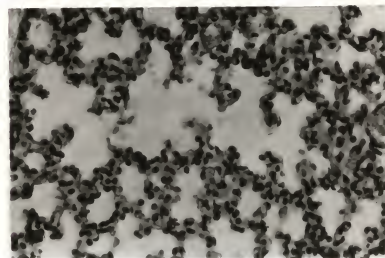


Fig 15-Apulmonary arteriole and secondary bronchiole are lying adjacent to each other. Note the wall of the bronchiole is lacking cartilage, but the lamina muscularis mucosae is present and continuous. The lamina propria is scant but consists of fine collagenous and elastic fibers. 76X-144 (near term); H & E stain.

Fig 16- This is another section of a pulmonary arteriole and bronchiole. 76X-111 (near term); H & E stain.

Fig 17-The pulmonary arteriole is alone in this section. However, the pulmonary artery and its peripheral subdivisions follow the distribution of airways to the level of respiratory bronchioles, where they continue as capillary beds associated with alveoli. 76X-111 (near term); H & E stain.

Fig 15

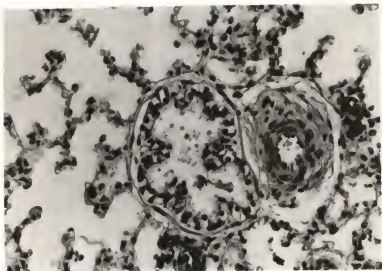


Fig 16

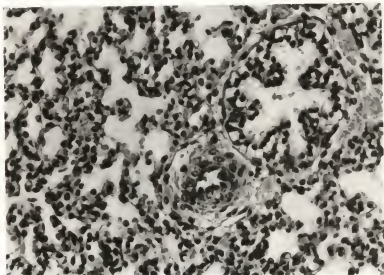


Fig 17

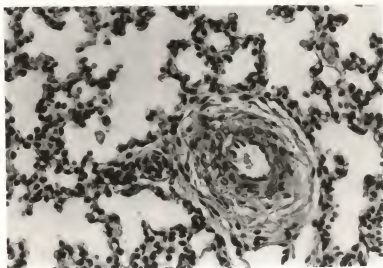


Fig 18-This is a tangential cut through a secondary bronchus. The lamina epithelialis is pseudostratified ciliated epithelium containing numerous goblet cells. The lamina propria is areolar connective tissue with numerous elastic fibers continuous with connective tissue of the hilus.

Fig 19-This is a cross-section of a large bronchus. Note the cilia and the cartilagenous plaque in the lower right. The lamina muscularis mucosae is present beneath the lamina propria. The tunica submucosa consists of areolar connective tissue and has branched, coiled tubuloalveolar mucous glands. 76X-144 (near term); H & E stain.

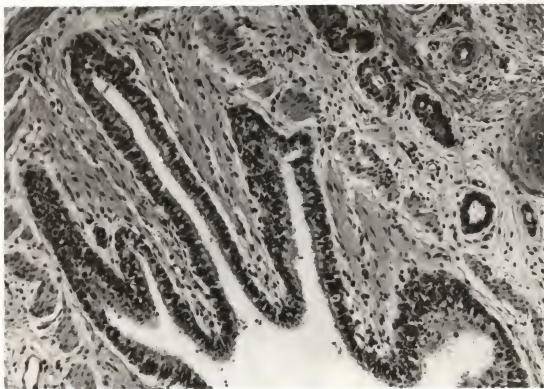


Fig 18

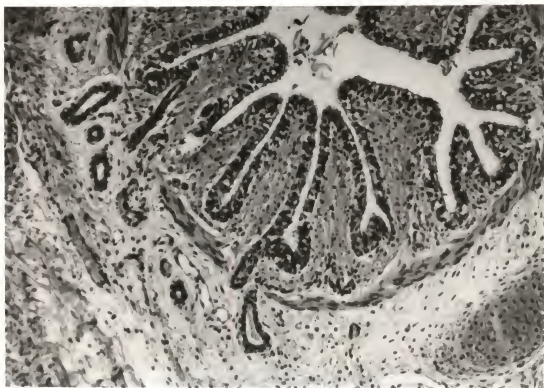


Fig 19

These photomicrographs demonstrate the lung capsule and its lining of visceral pleura.

The capsule consists of loose areolar connective tissue that is usually rich in elastic fibers. Note how interlobular septa are similar to and continuous from the capsular tissue. Blood and lymph vessels and nerves are present in the capsule.

A single layer of mesothelial cells covers the lung. This serous membrane follows the surface contours of lobes.

Note potential air spaces and thickness of their walls.  
Fig 20-76X-204 (3½ months); H & E stain.

Fig 21-76X-177 (7 months); H & E stain.

Fig 22-76X-224 (3½ months); H & E stain.

Fig 20

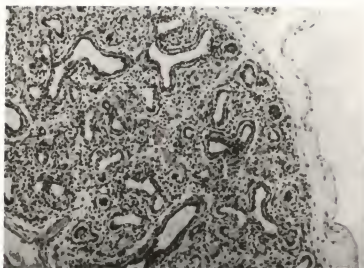


Fig 21

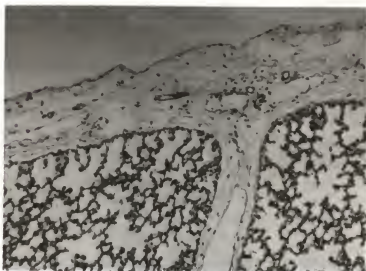
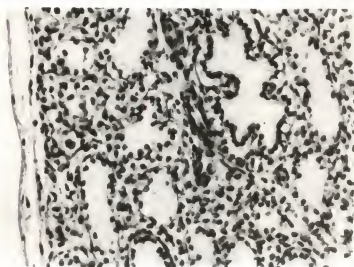


Fig 22





The next 6 photomicrographs illustrate various types of debris.

Fig 23-This bronchiole contains stringy, granular material and a few cells. This type of material was quite common. 76X-191 (near term); H & E stain.

Meconium was common within all air passages and alveoli. It is dark staining (arrows) and is closely associated with other material. Freshly aspirated meconium is usually golden-brown and easy to recognize.

Fig 24-76X-209 (5 months); H & E stain.

Fig 25-76X-209 (5 months); H & E stain.



Fig 23

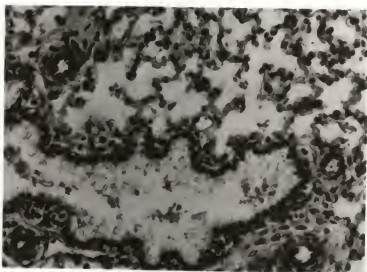


Fig 24

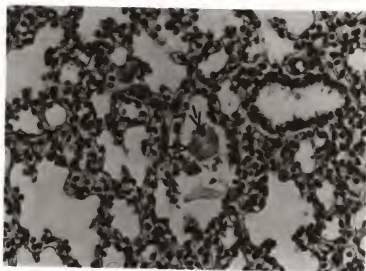


Fig 25

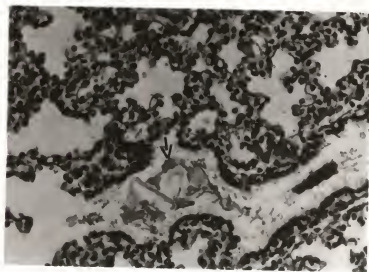


Fig 26-Two keratinized squamous cells (squamae) from the skin are associated with granular material and 2 clumps of meconium (m). 76X-225 (3½ months); H & E stain.

Fig 27-Note the typical aggregate of squamae. Clumps like this were common and seldom completely fill air spaces in normal fetuses. 76X-209 (5 months); H & E stain.

Fig 28-A single-celled, spiculed squame is present in an alveolar sac. The lower right bronchiole contains a clump of sloughed lining cells. 76X-155 (8 months); H & E stain.

Fig 26

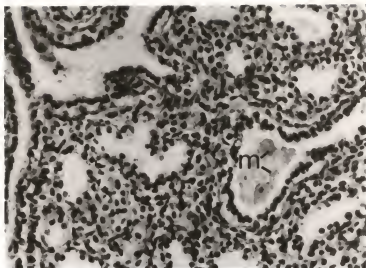


Fig 27

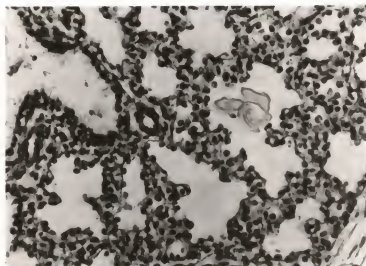
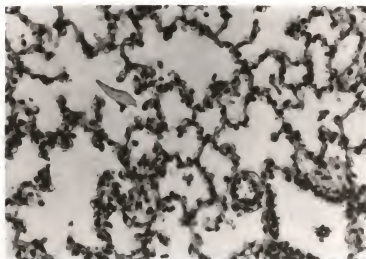


Fig 28



### III. HISTOLOGIC CHARACTERIZATION OF THE BOVINE FETAL LIVER

## INTRODUCTION

The liver is reported to be an organ unique to vertebrates and to have unequalled functional versatility. Embryologically, the liver is defined as a substitute yolk sac. Development occurs in close association with the yolk sac in all vertebrates and originated directly from the sac in Amphibia. Histologically the liver is described as a continuous muralium of parenchymal cells tunneled by capillaries that are lined by Kupffer cells. It conveys venous blood from the gastrointestinal system to the heart.<sup>1</sup>

Embryologic development and characterization of the mature liver has been reported often for various animal species; microscopic descriptions of development in the fetal bovine are lacking. Consequently, this investigation was concerned primarily with histologic description unique to the middle and late gestation in the bovine.

## MATERIALS AND METHODS

The methods were described in Part II of this thesis and in Appendix 1.

## RESULTS

The results from immunologic, bacteriologic, and histopathologic surveys on the fetuses are tabulated and summarized in Table I and in Appendix 2.

### Histologic Characterization:

A routine method of examining and evaluating fetal liver was

established. Results of microscopic features are tabulated in Table I and illustrated in Fig 1-17.

3½ to 5½ months gestation - Histologic features during this period were fairly consistent and straightforward. Liver sections were covered by a thin coat of peritoneum, and the capsule itself was usually a thin, loose connective tissue layer. Lobular architecture was not outstanding. Central veins were prominent in relationship to the portal areas, but were close together and randomly placed. Central veins were endothelium-lined spaces, usually containing blood cells, with scant amounts of perivascular connective tissue and not accompanied by ducts or arteries.

The portal areas contained bile ducts and ductules, a branch of the portal vein, a branch of the hepatic artery, lymphatic vessels, and nerves. These structures were surrounded by reticulum fibers, elastic fibers, and collagen fibers. The portal areas normally contained some histiocytes, but no lymphocytes or leukocytes. Some bile ducts were lined by low cuboidal epithelium, while others were lined by low columnar epithelium.

Parenchymal cells were polygonal and had reasonably distinct cytoplasmic membranes. The cytoplasm was generally amphophilic, ranging from more acidophilia to more basophilia, and was uniformly finely granular and lacy. Most hepatocytes had a single, large oval to round nucleus with a prominent nucleolus and little chromatin. Many megakaryocytes were present, but multinucleated hepatocytes and mitotic figures were rare. Liver cells were cordlike and radiated from central veins.

Sinusoids were identified but the space of Disse was not visible. The sinusoids were lined by flattened stellate cells with darkly

staining Kupffer cells occasionally projecting into the sinusoidal lumen. Entrance of sinusoids into central veins could usually be seen. Bile canaliculi were not observed.

Extramedullary hemopoiesis was extensive and scattered diffusely throughout the liver. The pattern varied from one or two cells to large aggregates distributed variably within the capsule, portal areas, sinusoids, and between hepatocytes.

6 to 7½ months gestation - This period was characterized by transitional rather than abrupt changes. The capsule remained a loose connective tissue layer that generally increased in thickness. Portal areas became more prominent and lobular architecture was slightly more distinct. Megakaryocytes decreased during this stage. The most dramatic change involved extramedullary hemopoiesis. It decreased and became focally intense but was scattered randomly throughout the liver.

8 months to near term - Lobular architecture was more consistent during this time, and the connective tissue within portal areas matured. Megakaryocytes continued to decrease and often were not observed. Hemopoiesis became indistinct and when observed, was always focal. As the fetus progressed to term, hepatocyte cytoplasm usually became more coarsely granular with concurrent droplets that were large and lipid-like.

#### DISCUSSION

Microscopic architecture in the bovine fetal liver did not change significantly from 3½ months to term. All structures were

easily identified at all developmental stages. The connective tissue matured and the lobules increased in size. Polygonal hepatocytes were generally consistent in shape and size. The cytoplasm was finely granular and became more vacuolated as development progressed to term. This phenomenon was also reported by Valdes-Dapena<sup>2</sup> and attributed to glycogen deposition. Megakaryocytes were prominent early and decreased significantly as term approached. Multinucleated hepatocytes were not common, a finding consistent with a previous report<sup>3</sup> that stated that binucleation increased after birth in rabbits, guinea pigs, cattle, and human. Hemopoiesis was altered and decreased by the seventh month. Along with decreased quantities of hemopoietic cells, patterns changed to more focally intense aggregates. Thomas et al<sup>4</sup> considered hepatic hemopoiesis involved erythropoiesis much more than granulopoiesis. To the diagnostician, however, differentiation between generalized hemopoiesis and inflammatory processes is more important. Portal areas became more prominent as gestation progressed; lymphocytes and leukocytes were not normal findings here. Fetal maturation of the liver was histologically apparent but not striking.

#### SUMMARY

After laboratory evaluation, 124 abattoir fetuses were deemed normal for histologic characterization. The fetuses varied in age from approximately 3½ months to near term. Histologic data of the fetal liver were remarkably consistent throughout the middle and late development. Essentially all microscopic structures were present to the end of the fetal periods, and tissue maturation and lobular sizes did increase. Significant alterations involved decline in megakaryocyte



number and hemopoietic cells around 7 months. Hemopoietic patterns also changed from diffusely scattered to focal aggregates about the same time. Over-all liver architecture of fetuses did not vary significantly from that expected in adult livers.

## REFERENCES

1. Elias H: Liver morphology. Biol Rev 30:263-310, 1955.
2. Valdes-Dapena MA: An Atlas of Fetal and Neonatal Histology. Philadelphia, J.B. Lippincott Co, 1957, pp 75-87.
3. Beams HW, King RL: The origin of binucleate and large mononucleate cells in the liver of the rat. Anat Rec 83:281-298, 1942.
4. Thomas DB, Russel PM, Yoffey JM: Pattern of hematopoiesis in fetal liver. Nature 187:876-877, 1960.



TABLE I: continued

| Fetal Age                           | 5 1/2 mo | 6 mo    | 6 1/2 mo | 7 mo    |         |
|-------------------------------------|----------|---------|----------|---------|---------|
| Case Number                         | 76X-203  | 76X-212 | 76X-215  | 76X-231 | 76X-141 |
| <b>Micronscopic Characteristics</b> |          |         |          |         |         |
| Visceral Peritoneum                 | +        | +       | +        | +       | +       |
| Fibrous Capsule                     | 3        | 3       | 2        | 1       | 3       |
| Hexagonal Lobular Structure         | Neg      | Neg     | Neg      | Neg     | Neg     |
| Acinar Interlobular Tissue          | 1        | 2       | 1        | 2       | 2       |
| Vasculature                         | +        | +       | +        | +       | +       |
| Nerves                              | +        | +       | +        | +       | +       |
| Extramedullary Hemopoiesis          | 2        | 4       | 3        | 4       | 2       |
| Megakaryocytes/LPF                  | 7        | 7       | 6        | 10      | 6       |
| Central Vein                        | +        | +       | +        | +       | +       |
| Hepatocytes                         | +        | +       | +        | +       | +       |
| Polygonal                           | +        | +       | +        | +       | +       |
| Amphophilic Cytoplasm               | +        | +       | +        | +       | +       |
| Vacuolization                       | 1        | 1       | 1        | 1       | 1       |
| Cord Arrangement                    | +        | +       | +        | +       | +       |
| Round Venicular Nuclei              | +        | +       | +        | +       | +       |
| Kupfer Cells                        | +        | +       | +        | +       | +       |
| Stellate                            | +        | +       | +        | +       | +       |
| Flattened Nuclei                    | +        | +       | +        | +       | +       |
| Sinusoids                           | +        | +       | +        | +       | +       |
| Portal Areas                        | +        | +       | +        | +       | +       |
| Portal Vein                         | +        | +       | +        | +       | +       |
| Hepatic Artery                      | +        | +       | +        | +       | +       |
| Bile Duct                           | +        | +       | +        | +       | +       |
| Lymphatics                          | +        | +       | +        | +       | +       |
| Nerves                              | +        | +       | +        | +       | +       |

TABLE 1: continued

[illegible]



These photomicrographs illustrate the liver parenchyma covered by the capsule (Glisson's capsule) and mesothelial layer of peritoneum. Extramedullary hemopoiesis is prominent as black speckling, and it diminishes as development progresses.

Fig 1-76X-206 ( $4\frac{1}{2}$  months); H & E stain.

Fig 2-76X-140 (6 months); H & E stain.

Fig 3-76X-139 (8 months); H & E stain.

Fig 1

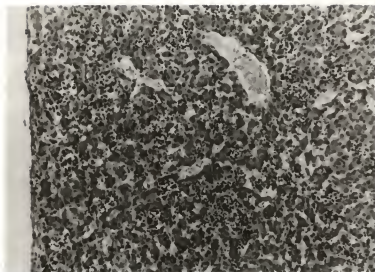


Fig 2

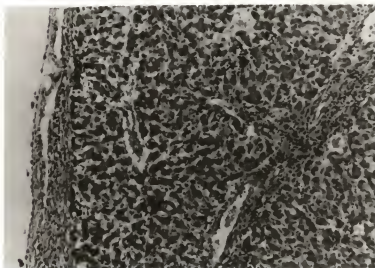
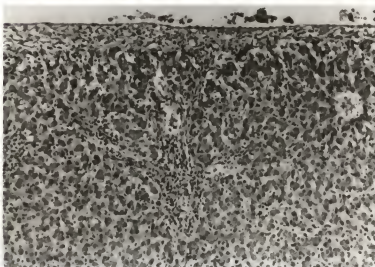


Fig 3





These are a higher magnification of the 3 previous photomicrographs.

Peritoneum is a single-cell layer lining the liver. The loose connective tissue of the capsule is well developed and is continuous with interstitial connective tissue.

Extramedullary hemopoiesis is evident as cells with small, dark, round nuclei with little cytoplasm. These cells usually lie within sinusoids, but may be associated with portal areas and the capsule.

Fig 4-Hemopoiesis is extensive and scattered diffusely throughout the section. Megakaryocytes (m) are present and occur with greatest frequency early in gestation. 76X-206 (4½ months); H & E stain.

Fig 5-Note the lymphatic vessel and area of extramedullary hemopoiesis within this capsule. 76X-140 (6 months); H & E stain.

Fig6-Connective tissue of Glisson's capsule is more dense here. The section is almost devoid of hemopoiesis. 76X-139 (8 months); H & E stain.

Fig 4

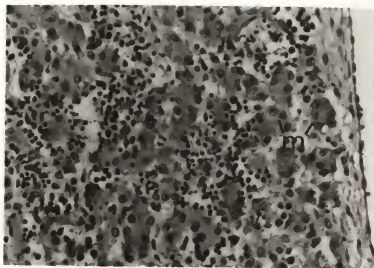


Fig 5

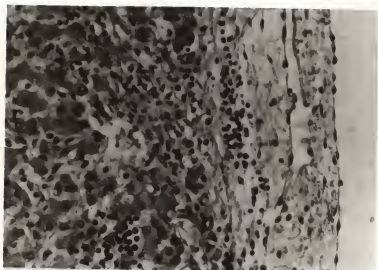
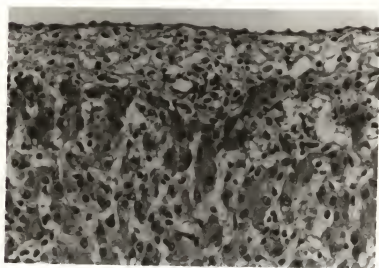


Fig 6



These photomicrographs illustrate prominent megakaryocytes. These giant cells were common during early stages of development, and the number decreased considerably during the 7th month.

Fig 7-76X-133 (7 months); H & E stain.

Fig 8-76X-139 (8 months); H & E stain.

Fig 9-76X-195 (near term); N & E stain.

Fig 7

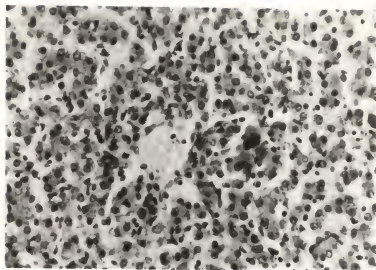


Fig 8

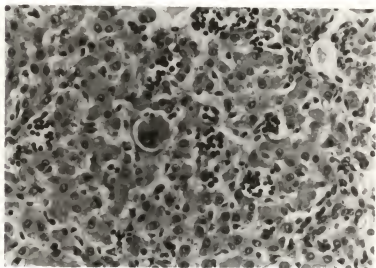
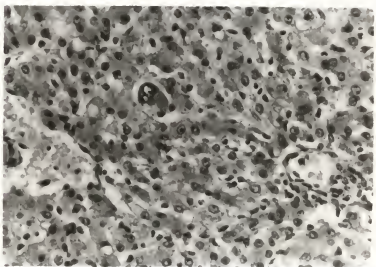


Fig 9



Portal areas are located between lobules and contain branches of the bile duct (b), portal vein (p), hepatic artery (a), and lymph vessels (l). Nerves are present but difficult to identify consistently. The structures are surrounded by reticulum fibers, elastic fibers, and collagen fibers.

Fig 10-Note the extramedullary hemopoiesis present in this portal area. 76X-195 (4½ months); H & E stain.

Fig 11-Cuboidal epithelium with prominent nuclei lining the bile duct; it is sometimes columnar. 76X-217 (5 months); H & E stain.

Fig 12-The fine, dark granules in this section are acid hematin. 76X-217 (5 months); H & E stain.

Fig 10

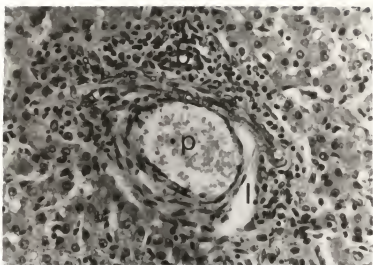


Fig 11

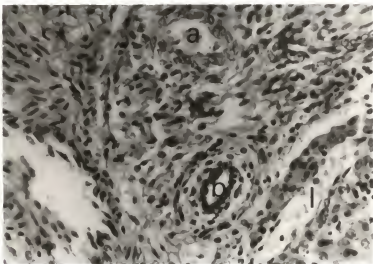
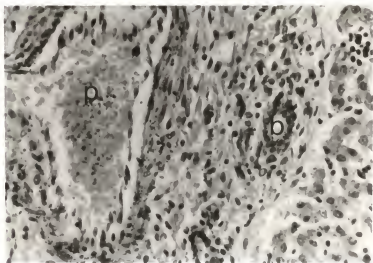


Fig 12



Two photomicrographs of sinusoids emptying into central veins. Central veins are lined by endothelium and usually contain blood cells.

Fig 13-Arrangement of liver cells is cord-like, and they radiate from central veins. Hepatocytes have large nuclei and finely granular cytoplasm. 76X-147 (8 months); H & E stain.

Fig 14-Note the prominent endothelium lining this central vein. 76X-134 (8½ months); H & E stain.

Fig 15-Cells making up walls of sinusoids are present. These Kupffer cells (k) are flattened and stellate. 76X-147 (8 months); H & E stain.

Fig 13

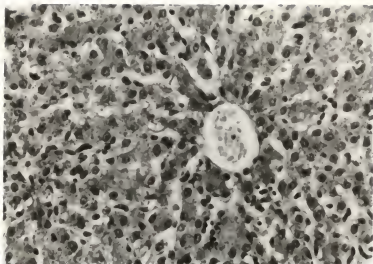


Fig 14

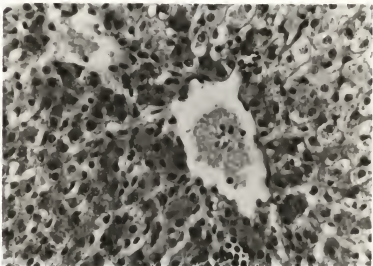


Fig 15

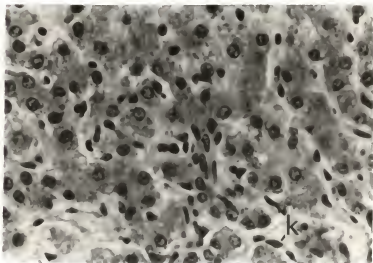




Fig 16-Megakaryocytes and abundant hemopoietic cells are prominent. 76X-206 (4½ months); H & E stain.

Fig 17-Generalized vacuolization of the cytoplasm of hepatocytes becomes more conspicuous as development progresses to term. The vacuoles have distinct borders, are clear, and vary in size. 76X-103 (near term); H & E stain.

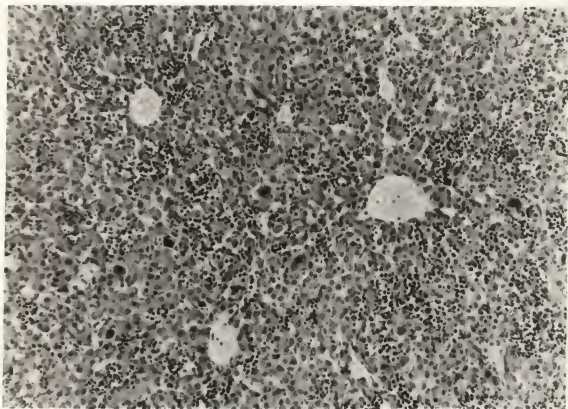


Fig 16

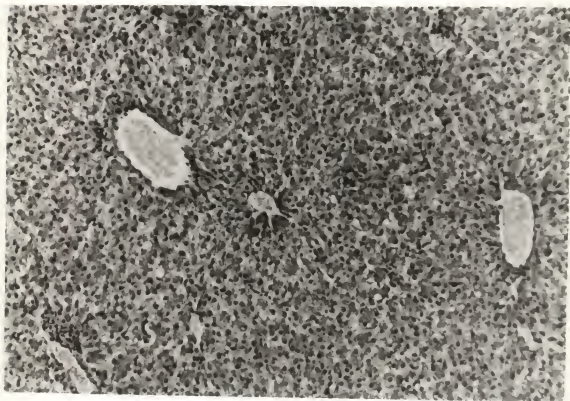


Fig 17

#### IV. HISTOLOGIC CHARACTERIZATION OF THE BOVINE FETAL KIDNEY

## INTRODUCTION

The kidney was no exception when information was gathered concerning development and histologic characterization of later fetal periods. Reports often involved only embryonic periods and detailed investigations into specific developmental abnormalities occurred.<sup>1,2</sup> The purpose of the study reported here was to describe the microscopic structures and features during the middle and late bovine fetal development.

## MATERIALS AND METHODS

The procedures followed were described previously in Part II of this thesis.

## RESULTS

Immunologic, bacteriologic, and histopathologic findings are summarized in Appendix 2.

### Histologic Characterization:

A routine method of examination of fetal kidney sections was established. The findings are summarized in Table 1 and illustrated in Fig 1-21.

3½ to 5½ months gestation - The most striking feature during this period was the prominent nephrogenic zone just beneath and parallel to the capsule. These zones had nephrogenic vesicles and ampullae located peripherally that differentiated to maturing renal corpuscles and

urinary tubules and the excretory system. Nephrogenic zones were mesenchymal in origin, they were deeply basophilic, and had numerous mitotic figures. Bowman's spaces were initially lined by columnar to cuboidal epithelium that progressively flattened to simple squamous. Parietal layers were always more flattened than visceral layers. Immature glomerular capillaries were lined by cuboidal epithelium, that tended to flatten as the glomeruli matured. Nephrogenic zones were most prominent up to  $4\frac{1}{2}$  months gestation and decreased slightly in activity and magnitude toward the end of this period.

Renal corpuscles were composed of tufts of capillaries that connected afferent and efferent arterioles that were surrounded by Bowman's capsules. Maturing Bowman's capsules, within the cortex deep to nephrogenic zones, usually had parietal, simple squamous epithelium and sometimes visceral, simple squamous epithelium. When visceral squamous epithelium was present, it was difficult to distinguish the visceral lining from capillary endothelium. Stellate mesangial cells were occasionally located between capillary loops of glomeruli and also within the juxtaglomerular apparatus of the vascular poles. Parietal, simple squamous epithelium gradually became cuboidal at the urinary poles, where it continued into proximal convoluted tubules. Renal corpuscles, including nephrogenic vesicles and maturing corpuscles, observed per low power field varied in number but demonstrated a trend that was consistent throughout this stage of development.

A loosely adherent capsule of dense, irregular connective tissue covered the kidney and some smooth muscle fibers were occasionally observed toward the parenchyma. Where visible, capsular thickness and connective tissue development were uniform. Trabeculae were absent,

but interstitial tissue consisted of reticular fibers around small vessels and the collecting duct system. Loose connective tissue was located around large blood vessels.

Uriniferous tubules were lined by simple epithelium but had different characteristics in each segment. Definitive identification of each segment was often difficult. However, basic judgements were made and segmental differences noted. These differences became more apparent as fetal age increased. Early in this period, differences between convoluted tubules and the excretory duct system were most evident, but differentiation into other segments soon became apparent.

Proximal convoluted tubules were lined by granular to vacuolated, pyramidal cells that nearly filled the lumen. Cell boundaries were indistinct and usually not visible. Each cell contained a single spherical nucleus irregularly located within eosinophilic cytoplasm. Brush borders on cell surfaces were not readily apparent. Proximal tubules made up the bulk of the kidney cortex.

Descending and ascending portions of the loops of Henle had cuboidal epithelium resembling proximal tubules. These segments were identified primarily by their presence within medullary rays. Thin segments of loops were lightly stained and contained flattened elongated cells with translucent cytoplasm and spheroid nuclei, that bulged into lumina. Thin loop segments were found deep in the medulla that often extended into medullary papillae. These segments were frequently arranged in nests within medullary mesenchyme and reticular stroma. Since numerous capillaries were present in the medulla, it was necessary to carefully distinguish the thin segments of loops of Henle.

Epithelium of distal convoluted tubules was lower and lumina were larger than the proximal counterparts. Although distal tubules were not as large as the proximal tubules, they were lined by more cells and their lateral borders were more clearly defined. In addition, these cells were less acidophilic than the proximal counterparts and had nuclei central to basal in location.

Connecting and collecting tubules of the excretory duct system had irregular cuboidal cells with central nuclei. Cell boundaries were distinct, and the cytoplasm was clear. Epithelial cells became columnar with eccentrically located nuclei in the larger tubules. Papillary duct and renal pelvis epithelium was transitional and had clear cytoplasm.

Golden-brown pigment was frequently located within tubular epithelium, particularly the distal tubules. This pigment was round and had distinct borders but varied in size. Tubules at the cortico-medullary junctions most often contained the pigment, but it was occasionally observed in other tubules throughout the sections. Small arterioles adjacent to pigment-laden tubular cells often had the same pigment within their walls. Pigment granules filled the cytoplasm but were not associated with other cellular changes. Although the degree of pigmentation varied and usually involved only a few random tubules, it was observed consistently as fetal age increased.

6 months to near term - Very few changes occurred as fetuses matured to term. Nephrogenic zones continued to become less obvious and were only occasionally present after 7½ months. Medullary rays became more prominent and tubular segments more readily differentiated.

Birefringent crystals were observed within tubules of 3 near term fetuses, but there were no associated changes. The crystals were not obvious without polarized light. They varied in size and appeared as clumps or rosettes.

## DISCUSSION

Microscopic patterns in the bovine fetal kidney were similar to those described in other mammals.<sup>1,2</sup> Although Gersh<sup>1</sup> believed the human fetal kidney to function as an excretory organ by the end of the third month, Ivermark<sup>2</sup> reported enzymatic localization differences between fetal and adult nephrons that indicated excretory function was not the same. Histologically, only juxtamedullary nephrons with large, maturing renal corpuscles were considered functional so early in fetal development. The nephrogenic zone was usually prominent during early fetogenesis but regressed significantly by midgestation. Renal corpuscles varied considerably as they matured, particularly the visceral and parietal layers of Bowman's spaces. The older the fetus, the greater the distance between the renal pelvis and subcapsular area where the nephrons originated. As a result, the point any loop of Henle reached was dependent upon kidney size when the nephron originated. Complete maturation, however, was not always attained until adult life.<sup>3</sup>

Capsular development was difficult to evaluate because it was often absent. Valdes-Dapena<sup>4</sup> reported fetal capsules were more easily stripped than those of adults, and that was compatible with the findings here. Capsules observed, however, always consisted of dense connective tissue.



Tubular segments of nephrons were as difficult to assess as those in adult kidney sections. Differences between segments were noted and characterized but they did not vary significantly from adult descriptions. Little significance was attached to the golden-brown pigment within tubular epithelium, as it was not associated with other cellular changes. No special procedures were utilized to identify the pigment. It was interesting that pigment was most often observed in the corticomedullary regions during the fetal period studied.

Birefringent crystals in this study were typical for those reported as oxalates.<sup>5</sup> Gopal et al<sup>6</sup> reported congenital anomalies or various other congenital lesions associated with 54 of 56 calves with renal oxalosis. In addition, oxalate crystals were reported in 53.3% of cases without etiologic diagnoses and in 66.7% of cases with anomalies.<sup>5</sup> Only 10.3% of near term and 2.4% of all fetal kidneys examined here had crystals, and none of the fetuses had significant laboratory findings. Schiefer and Moffatt<sup>5</sup> speculated that nearly 20% of all pregnancies may have renal oxalosis without causing fetal death. They felt, however, that crystals predisposed calves to postnatal diseases, viral or other infectious agents. Renal oxalosis in this investigation was probably not significant, but it was interesting that all fetuses involved were near term.

#### SUMMARY

One-hundred twenty-four normal bovine fetuses between 3½ months and near term were collected at an abattoir

Nephrogenic zones were prominent and consistently observed until 5½ months gestation. After this, the zones rapidly regressed

and were only occasionally observed after 7½ months. Nephrogenic vesicles and ampullae were present within mesenchymal tissue of these proliferating zones. Renal corpuscles in various stages of development were observed, with the most mature ones being present at the corticomedullary region. Tubules were identified but not significantly different from those of mature kidneys. Two features were found associated with the tubules. A golden-brown pigment within epithelial cells, unassociated with lesions, was readily observed. Little significance was placed on this pigment. Oxalate crystals were present within tubules of 3 near term fetuses. Little significance was placed on their presence also, as no other microscopic changes were present. Kidney section characterized in this study were similar to those reported for human fetuses.

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5. Schiefer B, Moffatt RE: Bovine abortion associated with renal oxalosis in the fetus. Can Vet J 15:57-65, 1974.
6. Gopal T, Leipold HW, Cook JE: Renal oxalosis in neonatal calves. Vet Pathol 15:519-524, 1978.



TABLE I: continued

[illegible]





These photomicrographs demonstrate the total width of kidney parenchyma, including capsule, cortex, and as much of the pyramid as possible. They are all at the same magnification.

Note the progressive increase in breadth of the cortex, as well as the entire organ. There is also an increase in the number of glomeruli, and the medullary rays become increasingly prominent. The medullary rays contain descending and ascending portions of the loops of Henle and the collecting ducts.

Nephrogenic zones (z) of the first 2 cases are apparent even at this power, just beneath and parallel to the capsules.

Fig 1-76X-225 (3½ months); H & E stain.

Fig 2-76X-219 (5 months); H & E stain.

Fig 3-76X-194 (8 months); H & E stain.



Fig 1

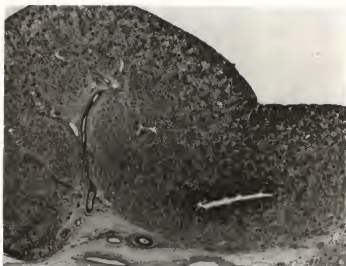
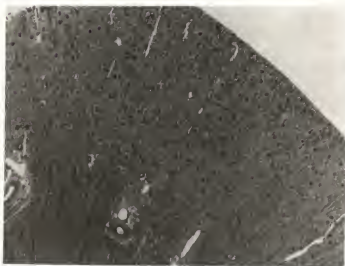


Fig 2



Fig 3



These photomicrographs illustrate the development of the renal cortex. Note the disappearance of a clear-cut nephrogenic zone by 7 months gestation. Glomeruli increase in size and distance between each other throughout gestation. The tubules constantly increase in number during development, and the proximal tubules make up the bulk of the renal cortex.

Fig 4-76X-219 (5 months); H & E stain.

Fig 5-76X-158 (7 months); H & E stain.

Fig 6-76X-194 (8 months); H & E stain.

Fig 4

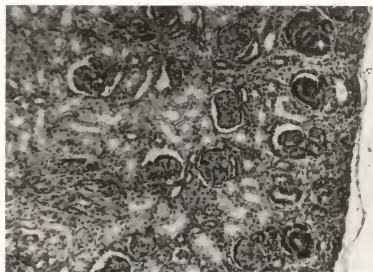


Fig 5

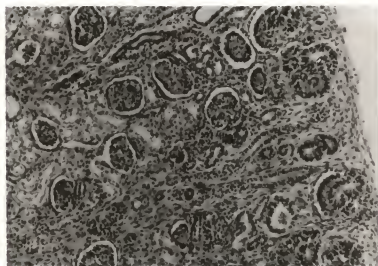
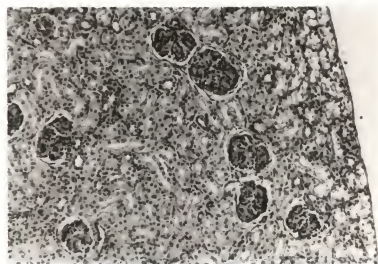


Fig 6



These photomicrographs are all from the same case and illustrate an active nephrogenic zone. Note the differences between visceral and parietal epithelium of Bowman's capsule. Fig 7-The lumina of the vesicles (V) broaden and basal cells become slightly less columnar. Most lower cells flatten and become precursor cells of Bowman's capsule. Some cells temporarily enlarge (arrows), that eventually become the epithelial portion of the glomerulus. Ampullae (A) divide into tubules. 76X-97 (4 months); H & E stain.

Fig 8-Cells of Bowman's capsule begin to flatten, and the ampullae (A) divide into tubules. Note that the epithelial cells of the glomerulus have large nuclei. A vesicle (V) is present, with enlarging cells (arrow). 76X-97 (4 months); H & E stain.

Fig 9-Primitive S-forms (S) may be seen. 76X-97 (4 months); H & E stain.

Fig 7

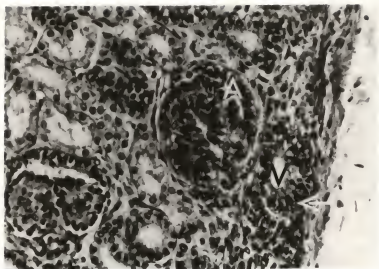


Fig 8

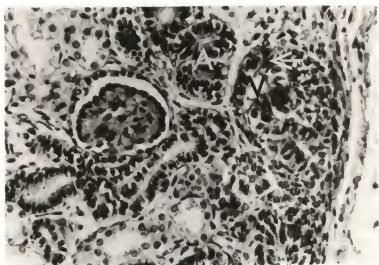
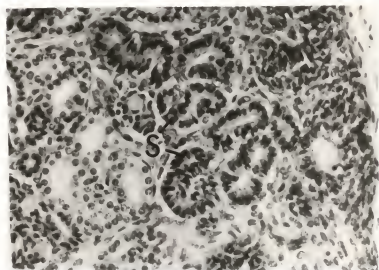


Fig 9



This series of photomicrographs illustrate the detailed changes in the subcapsular renal corpuscles.

The epithelium covering the glomerular capillaries and lining Bowman's capsule is striking. The parietal layer flattens to squamous epithelium early, while the visceral layer often remains cuboidal throughout later developmental stages.

Note how stromal tissue changes from mesenchymal to reticular.

As gestation progresses, there is an increase in the number of tubules.

Fig 10-76X-197 (4 months); H & E stain.

Fig 11-76X-207 (5 months); H & E stain.

Fig 12-76X-194 (8 months); H & E stain.

Fig 10

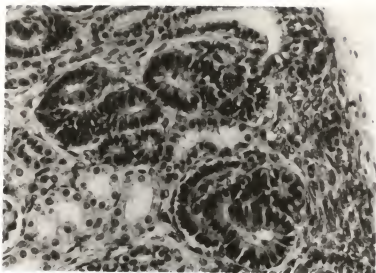


Fig 11

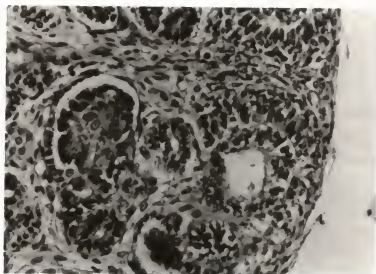
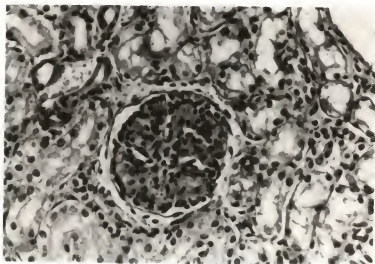


Fig 12



Changes in this group of juxtamedullary glomeruli are not nearly so striking as those in the subcapsular region. Probably the most significant feature is how the cuboidal epithelium covering the glomerular capillaries often flattens out later in gestation. This flattening was neither consistent nor obvious in all fetuses. Glomerular size may be significant in these cases.

Note the differences between proximal (p) and distal (d) tubules.

Fig 13-76X-197 (4 months); H & E stain.

Fig 14-76X-141 (5½ months); H & E stain.

Fig 15-76X-194 (8 months); H & E stain.



Fig 13

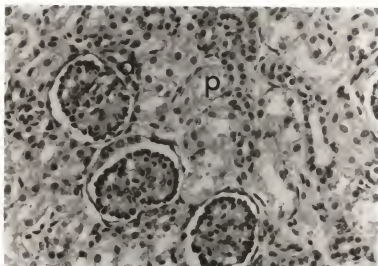


Fig 14

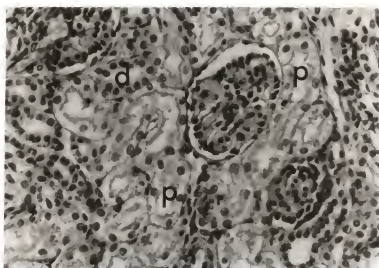
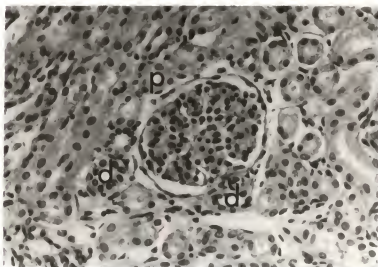


Fig 15



These photomicrographs were taken at the tip of pyramids. It is interesting to note an increase and maturity of interstitial tissue as development progresses.

During the early stages of gestation, transitional epithelium lines the renal pelvis and often lines the distal collecting ducts. Note differences in sizes of ducts.

Fig 16-Note how tubules are forming nests within the mesenchyme and reticular stroma. 76X-225 (3½ months); H & E stain.

Fig 17-Transitional epithelium is lining the renal pelvis and some ducts. 76X-141 (5½ months); H & E stain.

Fig 18-Note the similarity between capillaries (c) and thin segments of loops of Henle (h) deep in the medulla. 76X-105 (near term); H & E stain.

Fig 16

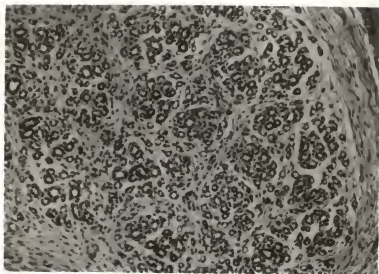


Fig 17

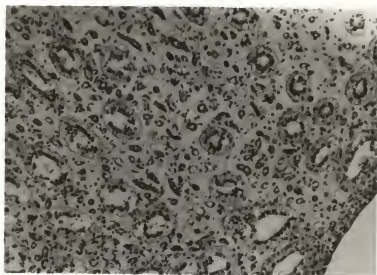
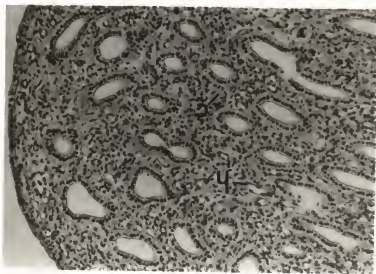


Fig 18



The first 2 photomicrographs were taken high in pyramids, to demonstrate the character of the epithelium lining collecting ducts. Note the prominent nuclei within clear cytoplasm.

Fig 19-76X-158 (7 months); H & E stain.

Fig 20-76X-105 (near term); H & E stain.

Fig 21-This is an example of the birefringent crystals present in tubules of near term fetuses (semi-polarized). There is no evidence of accompanying degeneration or necrosis. 76X-108 (near term); H & E stain.

Fig 19

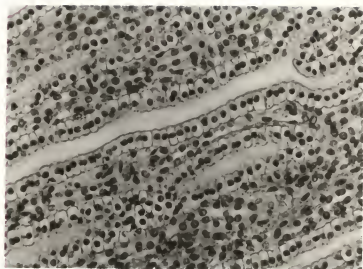


Fig 20

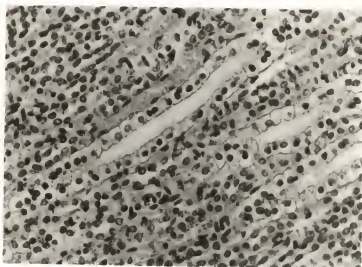


Fig 21



## APPENDIX

1. Procedures performed at South Dakota State University

## Necropsy Procedure

Each fetus was placed in lateral recumbency on its left side. A midline incision was made through the skin, extending from the mandibular symphysis to the pubis and the skin and right limbs reflected dorsally. The thoracic and abdominal cavities were opened by incising through the right costochondral junctions and the abdominal wall, respectively. Reflection of these allowed the thoracic and abdominal viscera to be examined in situ. The tongue was cut free of adjacent tissues and the hyoid bones disarticulated, allowing the tongue to be pulled postero-ventrally, so that the trachea and esophagus were freed from the adjacent cervical tissues. The tongue, larynx, esophagus, thymus, heart, and lungs were released upon incision of the mediastinum and thoracic-esophagus at the esophageal hiatus of the diaphragm. Abdominal viscera was removed by incising the abdominal esophagus at the esophageal hiatus of the diaphragm, the colon at the pelvic inlet, and the mesenteric attachments. Spleen, liver, and gallbladder were examined separately, and the urinary tract was removed intact.

Portions of lung, liver, spleen, and kidney were collected as aseptically as possible for bacteriologic examination and placed in sterile plastic bags. The abomasal wall was punctured with a sterile needle and a portion of the contents drawn into a sterile disposable syringe. Lung, liver, and kidney were placed in 10% buffered neutral formalin for histologic examination.

Blood was collected from the umbilical veins for immunoglobulin determination.



### Bacteriologic Examination

The portions of lung, liver, spleen, and kidney to be cultured were flamed, opened with sterile scissors, and smeared onto 5% sheep blood agar plates. A drop of abomasal contents was streaked on blood agar. The plates were incubated at 37 C in an atmosphere containing 10% CO<sub>2</sub> and were examined at 24 and 72 hours, and 7 days. In addition, a drop of abomasal contents was streaked on McConkeys agar and incubated aerobically. McConkey plates were also examined at 24 and 72 hours, and 7 days. When numerous similar colonies appeared, the organisms were isolated and biochemically characterized.

A drop of abomasal contents was placed on a slide, covered with a coverslip, and examined under darkfield illumination at X400.

### Radial Immunodiffusion

The fetal blood samples were allowed to clot and the sera stored at -20 C until tested. Radial immunodiffusion was performed with commercially prepared kitsets.\* To increase the sensitivity of the test for IgG concentrations less than 100 mg/100 ml, 5.0  $\mu$ l of serum or standard IgG solution was placed in each well rather than 2.5  $\mu$ l as recommended in the kit directions. IgM test kits were used as directed.

IgG solutions of known concentrations provided with the kits were diluted with buffered isotonic saline to produce standard solutions of 12.5, 25, 50, and 100 mg/100 ml, and the known IgM solutions were diluted to concentrations of 15, 30, 60, 130, and 250 mg/100 ml.

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\*files Laboratories, Kankakee, Ill.

The loaded IgG plates were incubated at room temperature for 18 hours and the IgM plates for 22 hours. Before measuring the precipitin rings, the plates were flooded 3 to 5 minutes with 1% tannic acid solution and then washed with a gentle stream of distilled water. Rings were examined with a magnifying glass using oblique light and a dark background. The diameters of the rings were read from the scale printed on the plates.

2. Results of immunologic, bacteriologic, and histopathologic findings of the fetuses

TABLE 1: Fetal Immunologic, Bacteriologic, and Histopathologic Findings

| Case Number | Fetal Age | IgM (ug/100ml) | IgG (ug/100ml) | Bacteriology                          | Histopathology           |
|-------------|-----------|----------------|----------------|---------------------------------------|--------------------------|
| 76X-198     | 3½ mo     | Neg            | Neg            | Enterica* & alpha-Streptococci        | NII                      |
| 76X-200     | 3½ mo     | ND             | ND             | alpha-Streptococci                    | NII                      |
| 76X-204     | 3½ mo     | Neg            | Neg            | Enterica & alpha-Streptococci         | NII                      |
| 76X-225     | 3½ mo     | Neg            | Neg            | Enterica                              | NII                      |
| 76X-197     | 4 mo      | Neg            | Neg            | Neg                                   | NII                      |
| 76X-199     | 4 mo      | Neg            | Neg            | Enterica & alpha-Streptococci         | NII                      |
| 76X-205     | 4 mo      | Neg            | Neg            | Few E. coli & alpha-Streptococci      | Lung-RBC Extravasation   |
| 76X-223     | 4 mo      | Neg            | Neg            | Neg                                   | Lung-RBC Extravasation   |
| 76X-226     | 4 mo      | Neg            | Neg            | Neg                                   | NII                      |
| 76X-224     | 4 mo      | Neg            | Neg            | Enterica                              | NII                      |
| 76X-227     | 4 mo      | Neg            | Neg            | Neg                                   | Lung-RBC Extravasation   |
| 76X-228     | 4 mo      | Neg            | Neg            | Neg                                   | NII                      |
| 76X-201     | 4½ mo     | Neg            | Neg            | Few Enterica & Few alpha-Streptococci | NII                      |
| 76X-206     | 4½ mo     | Neg            | 6-5            | alpha-Streptococci                    | NII                      |
| 76X-211     | 4½ mo     | Neg            | Neg            | Enterica                              | NII                      |
| 76X-210     | 4½ mo     | Neg            | Neg            | Neg                                   | NII                      |
| 76X-222     | 4½ mo     | Neg            | Neg            | Enterica                              | NII                      |
| 76X-229     | 4½ mo     | Neg            | Neg            | Enterica                              | NII                      |
| 76X-230     | 4½ mo     | Neg            | Neg            | Neg                                   | NII                      |
| 76X-163     | 5 mo      | Neg            | Neg            | Bacillus sp                           | NII                      |
| 76X-190     | 5 mo      | Neg            | Neg            | Neg                                   | NII                      |
| 76X-207     | 5 mo      | 88.0           | Neg            | Few alpha-Streptococci                | NII                      |
| 76X-208     | 5 mo      | Neg            | Neg            | Enterica & Proteus sp                 | NII                      |
| 76X-209     | 5 mo      | Neg            | Neg            | Few E. coli                           | Kidney-Mononuclear Focus |
| 76X-210     | 5 mo      | Neg            | Neg            | Few E. coli                           | NII                      |
| 76X-213     | 5 mo      | Neg            | 6-5            | Enterica                              | Lung-RBC Extravasation   |
| 76X-217     | 5 mo      | Neg            | Neg            | Enterica                              | NII                      |
| 76X-218     | 5 mo      | Neg            | Neg            | Enterica & alpha-Streptococci         | NII                      |
| 76X-219     | 5 mo      | Neg            | Neg            | Enterica & Proteus sp                 | NII                      |
| 76X-221     | 5 mo      | Neg            | Neg            | alpha-Streptococci                    | Lung-RBC Extravasation   |
| 76X-161     | 5½ mo     | Neg            | Neg            | Neg                                   | NII                      |
| 76X-203     | 5½ mo     | Neg            | Neg            | Enterica                              | NII                      |
| 76X-212     | 5½ mo     | Neg            | 150.0          | Enterica                              | NII                      |
| 76X-215     | 5½ mo     | Neg            | Neg            | Enterica & alpha-Streptococci         | NII                      |
| 76X-231     | 5½ mo     | Neg            | Neg            | Enterica                              | NII                      |
| 76X-160     | 6 mo      | Neg            | Neg            | Neg                                   | NII                      |
| 76X-164     | 6 mo      | 45.0           | 52.0           | Proteus sp                            | Lung-RBC Extravasation   |
| 76X-179     | 6 mo      | Neg            | 6-5            | Neg                                   | NII                      |
| 76X-187     | 6 mo      | Neg            | 18.0           | Few Enterica                          | NII                      |
| 76X-189     | 6 mo      | Neg            | 6-5            | Few Bacillus sp & Few Enterica        | NII                      |
| 76X-202     | 6 mo      | Neg            | Neg            | E. coli & alpha-Streptococci          | NII                      |
| 76X-172     | 6½ mo     | Neg            | 8.0            | Neg                                   | NII                      |
| 76X-182     | 6½ mo     | Neg            | Neg            | Few Enterica                          | Lung-RBC Extravasation   |
| 76X-188     | 6½ mo     | Neg            | 35.0           | 1. Staphylococcus sp & 1 Enterica     | NII                      |
| 76X-92      | 7 mo      | ND             | ND             | Neg                                   | NII                      |
| 76X-99      | 7 mo      | ND             | ND             | Neg                                   | NII                      |



TABLE I: Continued

| Case Number | Fetal Age | IgM (ug/100ml) | IgG (ug/100ml) | Bacteriology                    | Histopathology           |
|-------------|-----------|----------------|----------------|---------------------------------|--------------------------|
| 76X-98      | NT        | Neg            | Neg            | Neg                             | M11                      |
| 76X-100     | NT        | Neg            | 7.0            | Few Enterics                    | M11                      |
| 76X-101     | NT        | Neg            | 90.0           | Neg                             | M11                      |
| 76X-102     | NT        | Neg            | 55.0           | Few Pseudomonas sp              | M11                      |
| 76X-103     | NT        | Neg            | 8.0            | Neg                             | M11                      |
| 76X-104     | NT        | Neg            | 8.0            | Neg Enterics                    | M11                      |
| 76X-105     | NT        | Neg            | Neg            | Neg                             | M11                      |
| 76X-106     | NT        | Neg            | Neg            | Few Enterics                    | M11                      |
| 76X-107     | NT        | Neg            | Neg            | Neg                             | M11                      |
| 76X-108     | NT        | ND             | ND             | Few Enterics and Pseudomonas sp | M11                      |
| 76X-109     | NT        | Neg            | Neg            | Few Enterics and Bacillus sp    | Long-RBC Extravasation   |
| 76X-110     | NT        | Neg            | 8.0            | Few Enterics                    | M11                      |
| 76X-111     | NT        | Neg            | 13.0           | Neg                             | M11                      |
| 76X-112     | NT        | Neg            | 8.0            | Neg                             | Long-RBC Extravasation   |
| 76X-113     | NT        | Neg            | Neg            | Neg                             | M11                      |
| 76X-114     | NT        | Neg            | Neg            | Neg                             | Kidney-Homonuclear Focus |
| 76X-115     | NT        | Neg            | 150.0          | Neg                             | Long-Lymphoid Nodules    |
| 76X-116     | NT        | Neg            | Neg            | Neg Enterics                    | M11                      |
| 76X-144     | NT        | Neg            | Neg            | Neg                             | M11                      |
| 76X-148     | NT        | Neg            | 4.0            | Neg                             | M11                      |
| 76X-152     | NT        | Neg            | Neg            | Neg                             | M11                      |
| 76X-180     | NT        | Neg            | Neg            | Few Enterics                    | M11                      |
| 76X-183     | NT        | Neg            | Neg            | Neg                             | M11                      |
| 76X-185     | NT        | Neg            | 6.5            | Neg                             | M11                      |
| 76X-191     | NT        | Neg            | Neg            | Neg                             | M11                      |
| 76X-195     | NT        | Neg            | 10.0           | Few Enterics                    | M11                      |

\* Enterobacteriaceae, excluding E. coli

### Radial Immunodiffusion

Results of 117 abattoir fetuses are presented as immunoglobulin results were incomplete for 7 of the original 124 fetuses. Immunoglobulins were detected in sera from 54 (46.2%) of the 117 fetuses. Two fetuses demonstrated only IgM and 2 had both IgM and IgG levels, while 50 fetuses demonstrated only IgG levels. Thirteen (11.1%) fetuses had more than 20 mg/100 ml IgM, IgG, or both. The mean concentration of IgM was 55.9 mg/100 ml (range 0-88.0) and the mean concentration of IgG was 28.7 mg/100 ml (range 0-150.0)

### Bacteriology

Results of bacteriologic examination are listed in Table I. Bacteriologic results were not obtained for 2 fetuses. Bacteria were cultured from 66 (54.1%) of the 122 fetuses. Mixed bacterial cultures were isolated most often; 51 (41.8%) cultures were mixed and always included an enteric (Enterobacteriaceae excluding E coli) or alpha-hemolytic streptococci. Genera cultured alone and/or concurrent with others are listed: 49 (40.2%) enterics, 14 (11.5%) alpha-hemolytic streptococci, 8 (6.5%) Bacillus sp, 5 (4.1%) Staphylococcus sp, 4 (3.5%) Escherichia coli, 3 (2.5%) Proteus sp, and 2 (1.6%) Pseudomonas sp.

### Histopathology

Tissue changes were observed in 29 (23.4%) of the 124 fetuses, and 14 (48.3%) had concurrent immunoglobulin levels. Additionally, bacteria were cultured in 14 (48.3%) of the 29 fetuses. Red blood cell extravasation into lung parenchyma and interlobular septa was observed in 20 fetuses, while peribronchial lymphoid cuffs were noted only twice. An interstitial focus of mononuclear inflammatory cells was seen in 3

separate kidney sections. Two liver sections had portal areas infiltrated by mononuclear inflammatory cells and one section had extremely dilated central and portal veins. Results are tabulated with immunologic and bacteriologic findings in Table I.



HISTOLOGIC CHARACTERIZATION OF BOVINE  
FETAL LUNG, LIVER, AND KIDNEY

by

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AN ABSTRACT OF A MASTER'S THESIS

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The objectives of this study were to follow the histological development of the bovine lung, liver, and kidney through the middle and late fetal stages. Fetuses were collected from clinically healthy cows at slaughter in South Dakota and removed from the uterus within 30 minutes post mortem. Fetal specimens were also collected for bacteriologic, histopathologic, and radial immunodiffusion analysis.

Histologically, lung maturation was not uniform throughout the sections at any one fetal period. Organoid architecture gradually transformed from a mass of mesenchymal tissue with endodermal channels to a highly vascularized system of irregular air sacs lined by simple squamous epithelium. This transition was essentially complete at 7 months gestation. Capillaries migrated adjacent to air spaces, mesenchymal stroma decreased, and epithelium flattened as development progressed. Consequently, more complex saccular spaces were formed for efficient extrauterine respiration. Major architectural changes did not occur during the last 2 months. Lymphoid tissue was not observed around airways or vessels in any normal lung sections. Squamae and meconium were observed often, but little significance was placed on any debris unless it blocked air spaces. Early developmental stages of lungs coincided well with fetal age, and it is doubtful if independent bovine extrauterine existence is possible prior to 6 months gestation.

Histologic features in the liver did not alter as much as that of the lung. Microscopic structures were identified at all developmental stages. Cytoplasm of hepatocytes became more vacuolated near term and this was attributed to extensive glycogen depositions. Megakaryocytes were prominent early and were only occasionally observed after 7

months gestation. Hemopoietic activity decreased as development progressed and distribution became more focal after 7 months. Lymphocytes and leukocytes were not normally observed in portal areas. Architecture of fetal livers was remarkably consistent throughout middle and late gestation.

The most striking microscopic feature of kidney development was the nephrogenic zone beneath and parallel to the capsule. Nephrogenic vesicles and ampullae were active within the mesenchyme of the zone until midgestation. Although nephrogenic zones were not common after 7 months gestation, renal corpuscles continued to mature throughout gestation. Visceral and parietal layers of Bowman's capsule changed from cuboidal or columnar to simple squamous epithelium, but the parietal layer changed much earlier. Golden-brown pigment was often present within tubular epithelium. It was most common in distal convoluted tubules and the corticomedullary junctions, and special procedures were not done to identify the pigment. Birefringent crystals, believed to be oxalates, were present within the tubules. Little significance was placed on either of these findings because other microscopic changes were not present.

Fetal development and histologic characterization of bovine lung, liver, and kidney did not differ significantly from reports of other mammals. In addition to these observations, bacteriologic, immunologic, and histopathologic findings were made from this group of abattoir fetuses by the Animal Disease Research and Diagnostic Laboratory, South Dakota State University.